Application of Improved Technique in Tracing Karyotype Differences Between Strains of *Lathyrus odoratus* L.

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Received April 6, 1959

Introduction

The species of the genus *Lathyrus* L., have always been considered as favourable materials for cytological study. This is mainly because of the low number of chromosomes in the 2n complement as well as their medium size. Leaving aside the genetical work which was initiated at the time of Mendel, the species of this genus have also been explored from the cytological standpoint (Punnet 1925, Latter 1926, Roy 1936, Faberg 1935, Senn 1938, Larsem 1953, Bhattacharjee 1954, Datta 1955 etc. and vide Darlington and Wylie 1955).

It is well known that a number of horticultural varieties of *Lathyrus odoratus* L. have been raised from different centres. They mainly differ from one another with respect to colour of flowers together with certain other characters.

In spite of so much research having been carried out in different species of the genus, data regarding the details of the karyotypes are still lacking to a great extent. Leaving aside the different varieties of the same species, even some of the species have not been well explored as far as the chromosome morphology is concerned. Lately with the invention of a number of new methods involing pre-treatment in different chemicals new possibilities have been opened up in this line of investigation (Sharma 1956).

Considerable data have been gathered in the last few years with regard to the cytological basis of interstrain differences. Formerly most of the inter-strain variations were mainly attributed to gene mutations, or more precisely differences in the genic level which were cytologically undetectable. The development of such an idea has mainly been due to the non-availability of proper techniques, adequate enough to bring out minute karyotypic differences, if any, between different strains. Recent studies have shown that a number of cultivated crops as well as horticultural types show differences in chromosome morphology in different strains. For example in *Hordeum vulgare*, the common cultivated barley, different agricultural strains have been found to differ with respect to their karyotypes (Sharma and Mukherji 1956, Sharma 1957). Similar is the case with several cultivated strains of *Sorghum* (Sharma and Bhattacharjee 1957), *Triticum* (Bhaduri and Ghosh

1954) as well as *Secale* (Bose 1956). These studies, mainly aided with improved methods, clearly suggest that structural changes of chromosomes are also associated with the evolution of agricultural strains of the same species.

Lathyrus odoratus, as has been pointed out, contains a number of horticultural varieties. No work has yet been carried out to find out how far karyotypic changes or minute undetectable gene mutations have contributed to the origin of these varieties.

The solution to this problem obviously lies in the thorough analysis of the karyotypes of the different strains of *L. odoratus*, the common sweet pea. It is however to be admitted that any karyotypic difference, if found, does not necessarily mean that the same is solely to be considered responsible for interstrain differences in external phenotypic characters. Even then such studies provide at least important clues as to the problem of origin of the different varieties and strains of the same species. With this consideration in view, the present study deals with the karyotype analysis in details of the fourteen different varieties of *L. odoratus*.

Materials and methods

Fourteen different pure strains of *Lathyrus odoratus* L. were collected from a commercial Nursery of Calcutta. The varieties used in the present investigation are :—

1) "Blue Bird" with violet-blue flowers. 2) "Blue Danube" with lavender-blue flowers. 3) "Blue flakes" with blue flakes on white flowers. 4) "Chevalier" with rose-pink flowers. 5) "Daphne" with soft salmon-pink flowers. 6) "Early Marine" with early flowering clear blue flowers. 7) "Grey flakes" with grey flakes on chocolate-blue flowers. 8) "Harmony" with pale blue flowers. 9) "Lavender Glory" with lavender-coloured flowers. 10) "Mariner" with deep blue flowers (not early). 11) "Oriental" with deep primrose coloured flowers. 12) "Princess Blue" with rich belladonna blue large flowers. 13) "Princess Rose" with rosy white coloured flowers. 14) "Princess White" with pure white flowers which are yellow in the bud stage.

In addition to flower characters, the different varieties also differ in other morphological quantitative and qualitative features e.g. size and shape of leaves, colour of tendrils, colour of veins, thickness of leaves, nature of leaf surface, nature of stems etc.

For the study of somatic chromosomes of all the varieties mentioned above, seeds were germinated in separate pots in a mixture of clay, sand and sawdust. Several techniques involing prefixation with oxyquinoline (Sharma and Ghosh 1950), aesculin (Sharma and Sarkar 1955) and para-dichlorobenzene (Sharma and Mookerjea 1955) for varying periods, then fixing and hydrolysing by heating in a mixture of 2 % aceto-orcein solution

and normal hydrochloric acid in the proportion of 9:1 for about 3 seconds and finally smearing in 1 % aceto-orcein solution were tried. The best result was however obtained by prefixation with paradichlorobenzene for one hour only and then hydrolysing and smearing by the above-mentioned procedures.

Observations were made under oil-immersion lens with 1.3 o.e. apochromatic objective and ×12.5 eye-piece and the Camera lucida drawings were made with the same combination of lenses at a table magnification of ×3000 approximately.

Observations

General. Though all the varieties belong to a single species their chromosome complements are distinctly different in morphological nature.

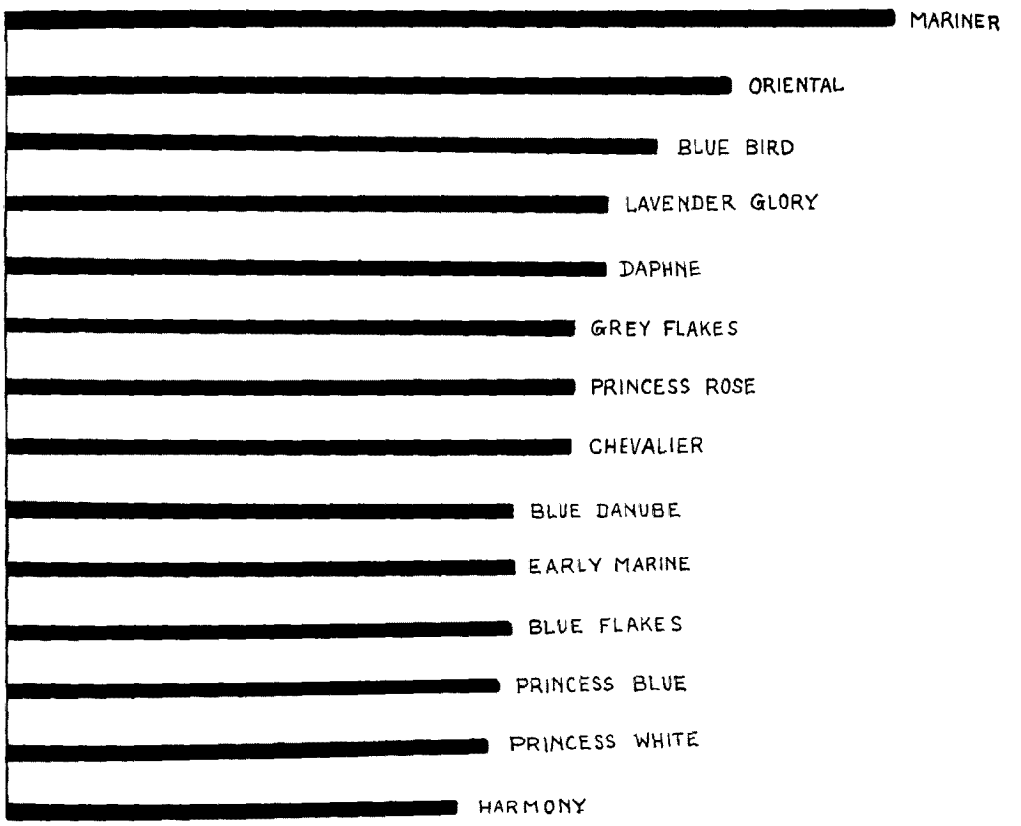


Fig. 1. Histogram of the amount of chromatin matter per length present in the haploid sets of the different varieties of *Lathyrus odoratus*.

The chromosomes possess median to submedian primary constrictions. Secondary constrictions are present at least in one pair of chromosomes in each complement. The positions of both the constrictions vary in different chromosomes of the same complement and in different varieties. As has been previously reported (Latter 1926, Datta 1955), the chromosome number in all varieties is $2n=14$.

The range of length of chromosomes in different varieties examined is 3.34 μ to 11.00 μ. The total amount of chromatin matter is slightly different in different varieties, the variety "Mariner" possessing the largest amount and the variety "Harmony" the smallest (Fig. 1). The number of secondary constrictions is highest in "Blue Bird" and lowest in "Chevalier", "Harmony" and "Princess White". The following table will indicate these features:—

Table 1.

	Variety	Total chromatin length (haploid)	Primary constrictions	Number of secondary constrictions in 2n set.
1.	Blue Bird	45.84 μ	median and submedian	eight
2.	Blue Danube	35.84 μ	median, submedian and subterminal	four
3.	Blue flakes	35.19 μ	median and submedian	six
4.	Chevalier	39.75 μ	median to nearly subterminal	two
5.	Daphne	42.50 μ	median and submedian	four
6.	Early Marine	35.84 μ	median and submedian	four
7.	Grey flakes	39.93 μ	median to nearly subterminal	six
8.	Harmony	31.83 μ	median and submedian	two
9.	Lavender Glory	42.66 μ	median to submedian	six
10.	Mariner	62.50 μ	median to subterminal	four
11.	Oriental	51.08 μ	median to submedian	four
12.	Princess Blue	34.50 μ	median to nearly subterminal	four
13.	Princess Rose	39.83 μ	median to submedian	four
14.	Princess White	34.00 μ	median to nearly subterminal	two

Size difference within a complement is not marked and the chromosomes from a more or less graded series. On the basis of their relative size and positions of primary and secondary constrictions, the chromosomes can be divided into the following distinct types (Fig. 2):

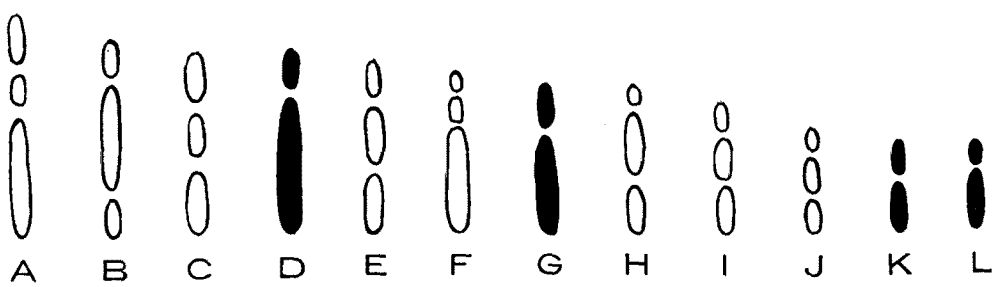


Fig. 2. Idiogram showing the different types of chromosomes present.

Type A (6.50 μ to 11.00 μ approx.): A long chromosome type with two constrictions, one primary and one secondary, situated very close together, one at nearly submedian and the other at submedian positions.

Type B (6.00 μ to 6.50 μ approx.): A long chromosome type with two

constrictions, primary and secondary, situated at submedian position at the two opposite ends of the chromosome.

Type C (6.50 μ to 8.50 μ approx.): A long to medium-sized chromosome type with two constrictions, one primary and one secondary, one nearly submedian and the other submedian in position.

Type D (4.50 μ to 9.50 μ approx.): A long to medium-sized chromosome type with a submedian primary constriction.

Type E (5.00 μ to 9.00 μ approx.): A long to medium-sized type of chromosome with two constrictions, primary and secondary, one nearly median and the other nearly submedian in position.

Type F (6.5 μ to 7.0 μ approx.): A long to nearly medium-sized chromosome type with two constrictions, primary and secondary, one nearly submedian in position and the other located in the middle of the shorter arm.

Type G (3.50 μ to 8.00 μ approx.): A medium-sized chromosome with submedian (or nearly subterminal) to nearly median primary constrictions.

Type H (6.00 μ to 6.60 μ approx.): A medium-sized chromosome with two constrictions, primary and secondary, one nearly submedian in position and the other subterminal at the distal end of the longer arm.

Type I (6.00 μ to 7.00 μ approx.): A nearly medium-sized chromosome with two constrictions, primary and secondary, one nearly submedian in position and the other located at the middle of the longer arm.

Type J (4.50 μ to 5.50 μ approx.): A nearly medium-sized to nearly short chromosome with two constrictions, primary and secondary, one median and the other submedian to nearly submedian in position.

Type K (3.34 μ to 6.00 μ approx.): Nearly short to short chromosomes with nearly median to nearly submedian primary constrictions.

Type L (3.50 μ to 4.00 μ approx.): Nearly short to short chromosomes with submedian to nearly subterminal primary constrictions.

On the basis of the above description of chromosome types, the karyotypes of each variety have been discussed. The detailed karyotype analysis for each variety is given below:

1) Variety "*Blue Bird*"

Slight size difference amongst the chromosomes of this variety is present which ranges from 5.35 μ to 8.50 μ approximately. The karyotype of this variety may be represented in the formula—2A, 6C, 2G, 4K. Out of these four types of chromosomes, two types (A and C) involving four pairs of chromosomes are with secondary constrictions. The chromosomes of this variety are broadly of the following classes (Figs. 3 and 4):

1. Four pairs of chromosomes with secondary constrictions, one of A and three of C types.

2. Three pairs of chromosomes with submedian primary constrictions, one of G and two of K types.

The different chromosome pairs have got different lengths e.g. A–6.67 μ, C–6.67 μ to 8.50 μ, G–6.50 μ and K, 5.35 μ approximately.

2) Variety "*Blue Danube*"

Slight size difference amongst the chromosomes of this variety is present which ranges from 4.00 μ to 6.67 μ approximately. The karyotype of this variety may be represented in the formula—2C, 2E, 4D, 2G, 4K. Out of these five types of chromosomes, two types (C and E) involving two pairs are with secondary constrictions. The chromosomes of this variety are broadly of the following classes (Figs. 5 and 6):

1. Two pairs of long to medium chromosomes bearing secondary constrictions, one of E and one of C types.
2. Two pairs of long to medium-sized chromosomes without secondary constriction which are of D type.
3. One pair of nearly medium-sized chromosomes of G type.
4. Two pairs of nearly short chromosomes of K type.

The different chromosome pairs have got different lengths: e.g. C–6.50 μ, E–6.67 μ, D–6.00 μ, G–5.20 μ, K–4.00 μ approximately.

3) Variety "*Blue Flakes*"

Slight size-difference amongst the chromosomes of this variety is present which ranges from 4.00 μ to 6.50 μ approximately. The karyotype of this variety may be represented in the formula—2D, 4E, 2J, 2K, 4L. Out of these five types of chromosomes, one type (E) involving three pairs possesses secondary constrictions. The chromosomes of this variety are broadly of the following classes (Figs. 7 and 8):

1. One pair of long chromosomes of D type.
2. Three pairs of long to medium-sized chromosomes with secondary constrictions, two of E type and one of J type.
3. Three pairs of nearly short chromosomes, one of K and two of L types.

The different chromosome pairs have got different lengths, e.g. D–6.50 μ, E–5.50 μ to 6.20 μ, J–5.00 μ, K, 4.34 μ, L–3.85 μ to 4.00 μ approximately.

4) Variety "*Chevalier*"

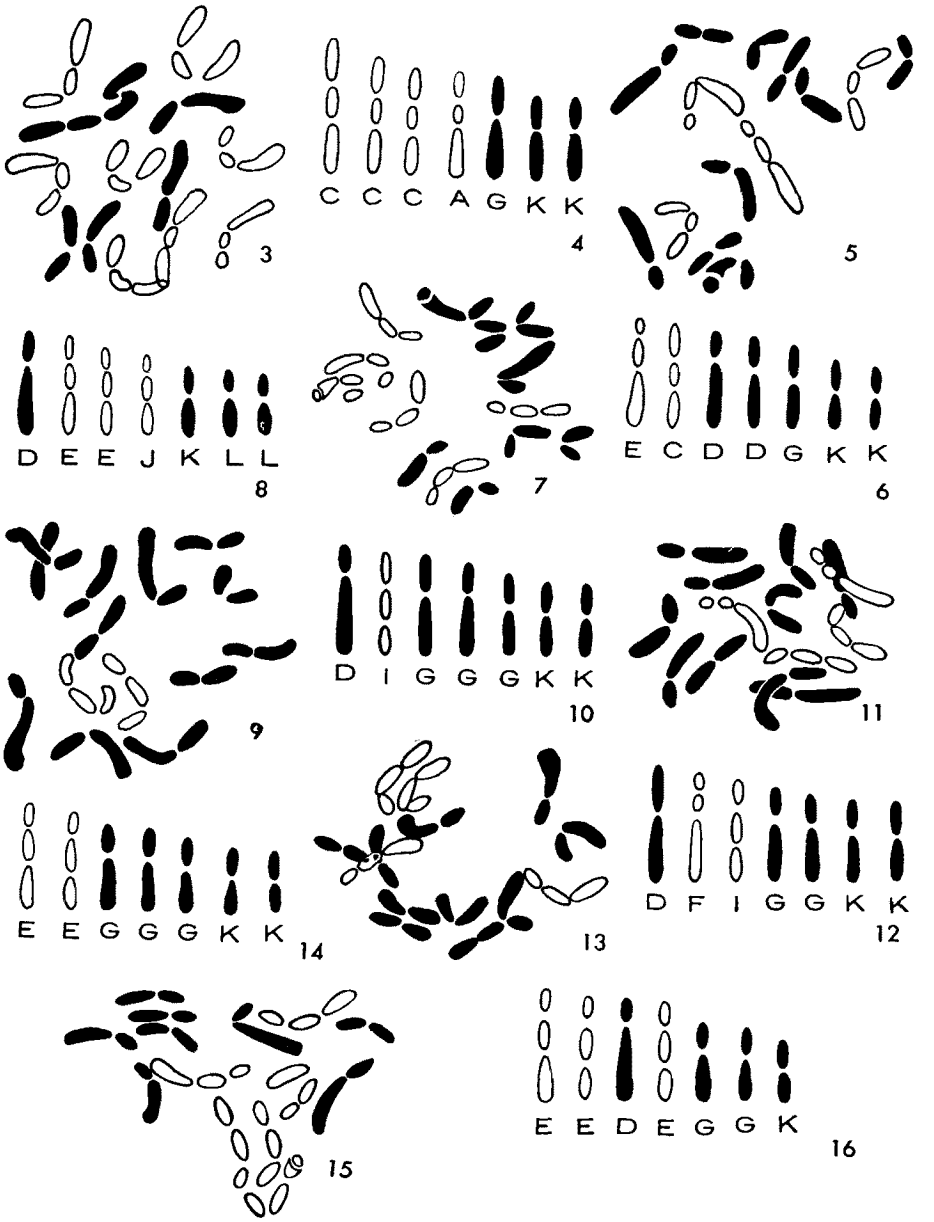
Slight size difference amongst the chromosomes of this variety is present, which ranges from 4.50 μ to 6.70 μ approximately. The karyotype of this variety may be represented in the formula—2D, 6G, 2I, 4K. Out of these four types of chromosomes, one type (I) involving one pair possesses secondary constrictions. The chromosomes of this variety are broadly of the following classes (Figs. 9 and 10):

1. One pair of long chromosomes of D type.
2. One pair of nearly medium-sized chromosomes with secondary constrictions of I type.
3. Three pairs of medium-sized chromosomes of G type.
4. Two pairs of nearly short chromosomes of K type.

The different chromosome pairs have got different lengths: e.g. D–6.70 μ, I–6.50 μ, G–6.67 μ to 6.50 μ, K–4.50 μ approximately.

5) Variety "*Dophne*"

Slight size difference amongst the chromosomes of this variety is present,



Figs. 3–16. 3 and 4, var. *Blue bird*—somatic metaphase plate and idiogram respectively. 5 and 6, var. *Blue Danube*—somatic metaphase and idiogram. 7 and 8, var. *Blue flakes*—somatic metaphase and idiogram. 9 and 10, var. *Chevalier*—somatic metaphase and idiogram respectively. 11 and 12, var. *Daphne* somatic metaphase and idiogram. 13 and 14, var. *Early Marine*—somatic metaphase and idiogram. 15 and 16, var. *Gray flakes*—somatic metaphase and idiogram.

which ranges from 4.00 μ to 7.00 μ approximately. The karyotype of this variety may be represented in the formula—2D, 2F, 2I, 4G, 4K. Of these five types of chromosomes two types (F and I) involving two pairs are with secondary constrictions. The chromosomes of this variety are broadly of the following classes (Figs. 11 and 12):

1. One pair of long chromosomes without secondary constriction of D type.
2. Two pairs of nearly medium-sized chromosomes with secondary constrictions, one of F and the other of I type.
3. Two pairs of medium-sized chromosome of G type.
4. Two pairs of nearly short chromosomes of K type.

The different chromosomes have got different lengths; e.g.—D-7.00 μ, F-6.85 μ, I-6.50 μ, G-5.80 μ to 6.00 μ, K-4.00 μ to 5.00 μ approximately.

6) Variety "*Early Marine*"

Slight size difference amongst the chromosomes of this variety is present, which ranges from 4.00 μ to 6.50 μ approximately. The karyotype of this variety may be represented as—4E, 6G, 4K. Out of these three types of chromosomes, one type (E) involving two pairs possesses secondary constrictions. The chromosomes of this variety are broadly of the following classes (Figs. 13 and 14):

1. Two pairs of long to medium-sized chromosomes of E type which are with secondary constrictions.
2. Three pairs of medium-sized chromosomes of G type.
3. Two pairs of nearly short chromosomes of K type.

The different chromosome pairs have got different lengths; e.g.—E-6.00 μ to 6.50 μ, G-4.70 μ to 5.30 μ, K-4.00 μ approximately.

7) Variety "*Grey flakes*"

Size difference amongst the chromosomes of this variety is present, which ranges from 4.20 μ to 7.00 μ approximately. The karyotype of this variety may be represented in the formula—2D, 6E, 4G, 2K. One type (E) involving three pairs is with secondary constrictions. The chromosomes of this variety are broadly of the following classes (Figs. 15 and 16):

1. Three pairs of long to nearly medium sized chromosomes of E type which bear secondary constrictions.
2. One pair of nearly medium sized chromosomes of D type.
3. Two pairs of medium sized chromosomes of G type.
4. One pair of nearly short chromosomes of K type.

The lengths of different pairs of chromosomes are—E-6.20 μ to 7.00 μ, D-6.40 μ, G-4.80 μ to 5.00 μ, K-4.20 μ approximately.

8) Variety "*Harmony*"

Size difference amongst the chromosomes of this variety is present which ranges from 3.34 μ to 6.00 μ approximately. The chromosomes of this variety may be represented in the formula—2B, 6D, 4G, 2K. Of these four types

of chromosomes, one type (B) involving one pair of chromosomes is with secondary constriction. The chromosomes of this variety are broadly of the following classes (Figs. 17 and 18):

1. One pair of long chromosomes bearing secondary constrictions of B type.
2. Three pairs of long to medium-sized chromosomes of D type.
3. Two pairs of medium-sized chromosomes of G type.
4. One pair of more or less short chromosomes of K type.

The different chromosome pairs have got different lengths, e.g. B–6.00 μ, D–4.50 μ to 5.50 μ, G–3.90 μ to 4.00 μ, K–3.34 μ approximately.

9) Variety "*Lavender Glory*"

The chromosomes of this variety differ slightly in size, which ranges from 4.00 μ to 6.70 μ approximately. The chromosomes may be represented in the formula—2C, 2E, 2H, 6G, 2K. Out of these, three types (C, E, and H) involving three pairs are with secondary constrictions. The chromosomes are broadly of the following classes (Figs. 19 and 20):

1. Three pairs of long chromosomes with secondary constrictions, of which one pair is of C type, one of E type and one of H type.
2. Three pairs of medium-sized chromosomes of G type.
3. One pair of nearly short chromosomes of K type.

The different chromosome pairs have got different lengths, e.g.—C–6.70μ, E–6.70 μ, H–6.60 μ, G–5.70 μ to 6.20 μ, K–4.00 μ approximately.

10) Variety "*Mariner*"

The chromosomes of this variety differ slightly in size, which ranges from 7.70 μ to 11.00 μ approximately. The chromosomes may be represented in the formula—2A, 2D, 2E, 8G. Out of these four types of chromosomes, two types (A and E) involving two pairs are with secondary constrictions. The chromosomes are broadly of the following classes (Figs. 21 and 22):

1. Two pairs of long to medium-sized chromosomes with secondary constrictions, one of A and one ot E types.
2. One pair of long chromosomes without secondary constriction of D type.
3. Four pairs of medium-sized chromosomes of G type.

The lengths of different chromosomes are—A–11.00 μ, D–9.50 μ, E–9.00μ, G–7.70 μ to 9.00 μ approximately.

11) Variety "*Oriental*"

The chromosomes of this variety differ slightly in size, which ranges from 6.00 μ to 9.00 μ approximately. The chromosomal complement may be represented in the formula: 4E, 8G, 2K. Out of these three types of chromosomes, one type (E) involving two pairs of chromosomes possesses secondary constrictions. The chromosomes are broadly of the following classes (23 and 24):

1. Two pairs of long to medium-sized chromosomes of E type with secondary constrictions.
2. Four pairs of medium-sized chromosomes of G type.
3. One pair of nearly short chromosomes of K type.

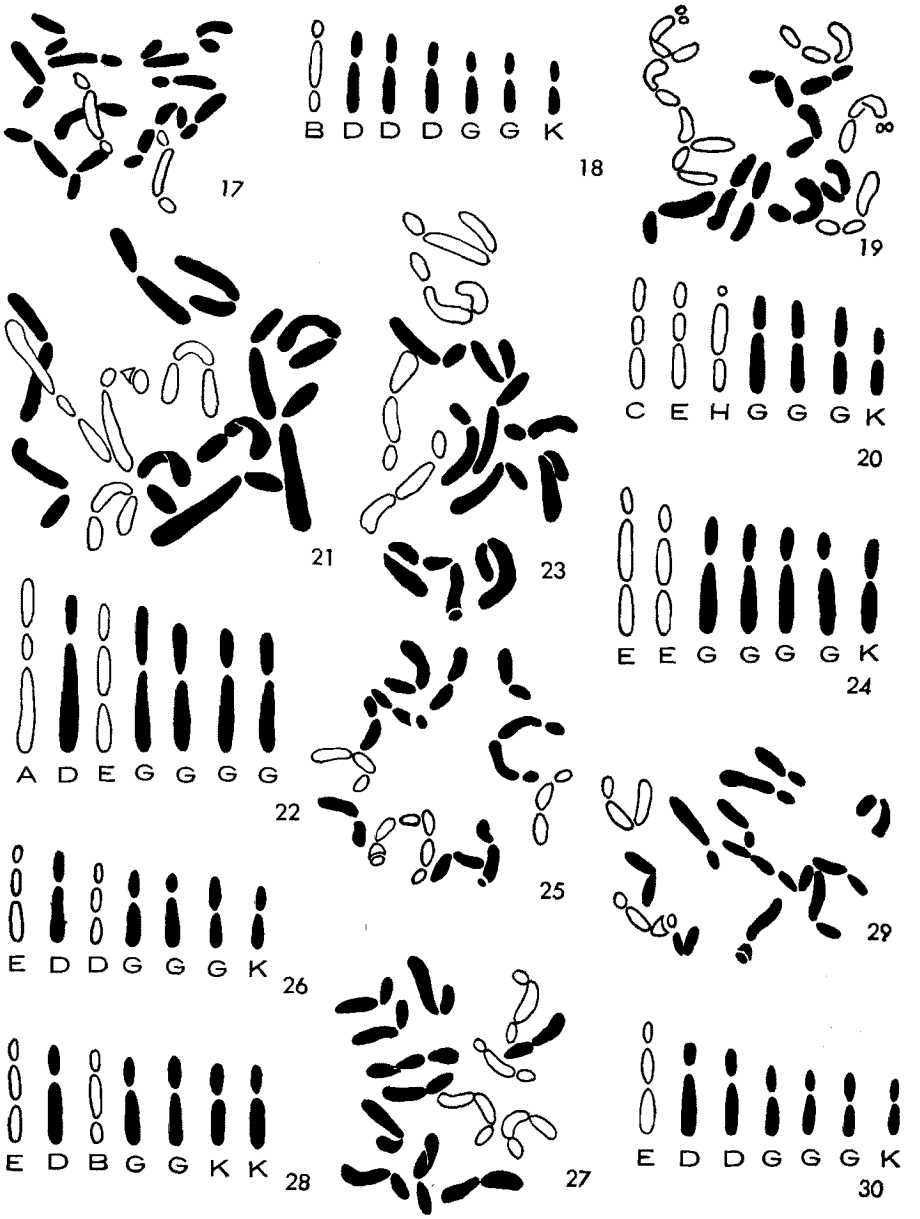


Fig. 17–30. 17 and 18, var. *Harmony*—somatic metaphase and idiogram. 19 and 20, var. *Lavender Glory*—somatic metaphase and idiogram. 21 and 22, var. *Mariner*—somatic metaphase and idiogram. 23 and 24, var. *Oriental*—somatic metaphase and idiogram. 25 and 26, var. *Princess blue*—somatic metaphase and idiogram. 27 and 28, var. *Princess rose*—somatic metaphase and idiogram. 29 and 30, var. *Princess white*—somatic metaphase and idiogram respectively.

The different chromosome pairs possess different lengths, e.g.—E–8.00 μ to 9.00 μ, G–6.67 μ to 7.67 μ, K–6.00 μ approximately.

12) Variety "*Princess Elue*"

The chromosomes of this variety differ slightly in size, which ranges from 4.00 μ to 6.00 μ approximately. The chromosomal complement may be represented in the formula—2D, 2E, 2I, 6G, 2K. Out of these five types of chromosomes, two types (E and I) involving two pairs are with secondary constrictions. The chromosomes are broadly of the following classes (Figs. 25 and 26):

1. Two pairs of long to medium-sized chromosomes of E and I types which bear secondary constrictions.
2. One pair of long chromosomes of D type.
3. Three pairs of medium-sized chromosomes of G type.
4. One pair of short chromosomes of K type.

The lengths of different chromosome pairs are—E–6.00 μ, D–5.85 μ, G–4.50 μ to 4.70 μ, I–5.00 μ, K–4.00 μ approximately.

13) Variety "*Princess Rose*"

The range in lengths of chromosomes of this variety is 5.50 μ to 6.50 μ approximately. The chromosomal complement may be represented in the formula—2B, 2D, 2E, 4G, 4K. Of these five types of chromosomes, two types (B and E) are nucleolar with secondary constrictions. The chromosomes are broadly of the following classes (Figs. 27 and 28):

1. Two pairs of long to medium sized chromosomes with secondary constrictions, one of B and one of E types.
2. One pair of long chromosomes of D type.
3. Two pairs of medium-sized chromosomes of G type.
4. Two pairs of nearly short chromosomes of K type.

Different pairs of chromosomes have got different lengths e.g. B–6.40 μ, D–6.40 μ, E–6.50 μ, G–5.70 μ, K–5.50 μ approximately.

14) Variety "*Princess White*"

The range in lengths of chromosomes of this variety is 3.34 μ to 6.70 μ approximately. The chromosomal complement may be represented as—4D, 2E, 6G, 2K. Out of these four types of chromosomes—one type (E) bears secondary constrictions. The chromosomes are broadly of the following classes (Figs. 29 and 30):

1. One pair of long chromosomes of E type bearing secondary constrictions.
2. Two pairs of mainly median to long chromosomes of D type.
3. Three pairs of medium-sized chromosomes of G type.
4. One pair of short chromosomes of K type.

Different chromosome pairs have got different length, e.g. D–5.70 μ to 6.00 μ, E–6.70 μ, G–4.00 μ K–3.34 μ approx.

Discussion

1. Karyotype as a distinguishing criterion for different strains

The present work on different strains of *Lathyrus odoratus* brings out clearly a number of interesting features as regards their karyotypes. Evidences have been brought forward to show that the strains can be distinguished on the basis of the chromosome complements.

A general homogeneity between different strains is established by the gross morphology of the chromosome complements. All of them are characterized by chromosomes varying from long to short in size, forming a graded series. No marked size difference is present and gradation is gradual, not abrupt. So far as the total length of chromatin matter per haploid complement is concerned, uniformity is also noticeable (vide Histogram). The differences in the total chromatin length exhibited between strain to strain are not very marked. The primary constriction mainly range from median to submedian in position. The secondary constrictions too vary similarly.

Inspite of the general homogeneity, a strain is characterised by its own karyotype. A thorough analysis of chromosome complements indicates differences between one strain and another, clearly brought out with the pretreatment chemicals applied here. In order to maintain a uniformity, pretreatment procedure for all the strains had been kept constant.

Strains mainly differ with respect to the number and position of constriction regions in the chromosomes. For example in strain "Blue Bird" eight chromosomes have been found to possess secondary constrictions. Whereas the other extreme is exhibited by the strain—"Princess white", where only two chromosomes with secondary constrictions are present. Intermediate conditions with respect to this character are represented in other strains. In addition to the differences in number and nature of chromosome types with secondary constrictions, other chromosomes too show variation from one strain to another. All these characters taken together indicate that so far as these strains are concerned, they can easily be identified on the basis of their karyotype.

2. Role of structural changes of chromosomes in evolution of strains

Such differences between one strain and another in minute karyotypic details indicate the role of structural changes of chromosomes in the evolution of different strains of *L. odoratus*. There is no reason to assume that they have mainly been evolved through gene mutations involving flower colour.

If at all it is assumed that structural alterations have been an associated feature during evolution of the strains, the problem may be raised as to why all of them breed true and also show regular behaviour. Regular meiosis has been noted in all these cases. A possible explanation of this behaviour can however be offered by assuming if one takes into consideration that they have undergone intensive cultivation and selection by the horticulturists.

Because of continued cultivation and selection structural alterations have all attained homozygosity. These strains may therefore be considered as all possibly homozygous for translocation. This is made however mainly on the basis of minor karyotypic differences and is yet to be substantiated through cross-breeding experiments. Cross-breeding between different strains may bring out heterozygous combination yielding expected configurations in meiosis and thus providing adequate proof of the assumption outlined above.

On the basis of the number of secondary constrictions it is not possible to state as to which of the strains represents a primitive condition. It may apparently seem that strains showing large number of secondary constrictions have possibly evolved from those with lesser number of constrictions in them. On the other hand, one cannot preclude this possibility that the lower number of secondary constrictions may have been evolved through loss of certain segments by amphiplasty during structural alterations. In view, however, of the data available on the plant genera (De Mol 1928, Chakravorti 1951), it appears that the strains with more secondary constrictions are comparatively more evolved than those with less number of constrictions in them.

Summary

In order to find out how far karyotypic changes or undetectable gene mutations have been associated with the origin of different varieties of *Lathyrus odoratus*, which differ in flower colour, leaf nature, tendril character etc., a thorough analysis of the karyotype of different pure varieties of *L. odoratus* have been carried out in the present investigation. For this purpose a new improved method involving pre-treatment with paradichlorobenzene was employed and the procedure for all the strains had been kept constant.

It has been revealed, that in spite of a homogeneity amongst all the strains, each strain is characterised by its own karyotype. They mainly differ with respect to the number and position of constriction regions in chromosomes. All these facts suggest that the structural changes of chromosomes have always been associated with the evolution of different varieties.

Regular meiosis noticed in these strains can be explained by assuming that through extensive cultivation and selection all the structural alterations have attained a state of homozygosity.

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